

May 23, 1951

Dr. Alex B. Novikoff
Department of Pathology
University of Vermont
Burlington, Vermont

Dear Alex:

I will try to answer your questions as they appeared in your letter:

(1) I will send you about 200 grams of dried Anheuser-Busch brewers' yeast. This was prepared especially for us and involved thorough washing of beer yeast followed by gentle drying. We obtained 100 pounds of this three years ago, and as we still have several pounds left, you are welcome to this sample. Other yeasts may do as well, but our experience with this method is limited to this particular yeast.

(2) The calcium phosphate gel step, as is typical of gelladsorptions, is excellent but finicky. I must confess that with a given gel we do not get consistent results over a period of time, very likely as a result of aging of the gel or perhaps minor variations in the salt content of the material to be adsorbed. For that reason, we always carry out trial adsorptions and elutations (with our own methods as well as those described by others) before proceeding to the larger scale operation. The last time we

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prepared Zwischenferment by the method described in JBC 182, 806, we found it necessary to modify the calcium phosphate gel step as follows:

Starting with 1200 cc. of the ammonium sulfate fraction, we added 200 cc. of calcium phosphate gel and discarded the precipitate. To the supernatant, which contained 91% of the activity, we then added another 140 cc. of gel which now adsorbed all but 60% of the activity. The gel was washed with water (about 100 cc.) and then with two 150 cc. portions of 0.5 M potassium phosphate buffer, pH 7.7. The eluate (300 cc.) was treated with 150 grams ammonium sulfate and the collected precipitate dissolved in water to 100 cc.

This material should be useful as is. There have been occasional difficulties in obtaining a good isoelectric precipitate. In the last preparation, we obtained material of very high potency in good yield by the following procedure:

To the ammonium sulfate fraction (100 cc.) were added 6.5 cc. of 5 M acetate buffer, pH 4.5, adjusted to pH 4.0 with 0.15 M acetic acid and finally diluted with water to 1 liter. The precipitate was collected on the centrifuge, dissolved with 40 cc. of 0.5 M glycylglycine buffer, pH 7.4, and lyophilized.

(3) The methods for preparing the gels are, so far as I can see, as described by Keilin and Hartree for calcium phosphate gel and by Willstätter and Kraut, Ber. 56, 1117 (1923) for C ✓ gel.

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(4) Mr. Dan Broida of Sigma was around the other day and said he would send us some of their TPN preparations for assay. I shall be glad to let you know how good they are, although there is no assurance that they will stay that way. We would be very happy, for our own sake, if we were relieved of the nuisance of having to prepare TPN and, even worse, trying to find sheep livers in this area.

I do not know whether it is necessary to emphasize that you should set up the assay for Zwischenferment in order to make good preparations of it. If you have some trouble in locating glucose-6-phosphate (the Schwarz product is about 60% pure and useful), let me know and perhaps we can help you out. Write me if any of these directions give you trouble.

With best regards,

Sincerely yours,

Arthur Kornberg

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